REMARKS

Applicants have amended claims 32-45 to include methods for identifying single nucleotide polymorphisms (SNPs) in the genome of mammals. However, Applicants reserve the right to prosecute the subject matter canceled in the claims in a continuation or divisional application. New claims 46-55 are directed to methods for identifying single nucleotide polymorphic sites in different types of mammals including human, non-human primates, dogs, cats, cattle, sheep and horses. These embodiments derive from Applicants' discovery of the distribution and density of SNPs in a mammalian genome and their penetrating insight that combining SNPs would provide valuable genetic information. Applicants, in their specification, teach and enable the advantageous properties of SNPs for genetic analysis in, among other things, mammals. See the specification, for example, at pages 13-15, page 44, lines 29-36 and Examples 1-6. Moreover, Applicants teach and enable using the combinations of SNPs, even at high allelic frequencies, to provide highly accurate genetic analysis. See the specification, for example, at pages 38-42, Examples 1-6 and Figures 4 and 5. No new matter has been added. Applicants respectfully request entry of this amendment and reconsideration of the application.

Supplemental Oath and Declaration

The Examiner alleged that the preliminary amendment filed May 22, 2002 introduced new matter requiring a supplemental oath or declaration. Applicants respectfully disagree with the Examiner's position. Nevertheless, Applicants have amended the claims to include methods for identifying single nucleotide polymorphic sites in the genome of mammals. Applicants respectfully submit that the claims as amended do not introduce new matter and respectfully submit that this requirement is moot in light of the amendments to the claims.

Objection to the Specification

The Examiner has objected to the specification under 35 U.S.C. § 132 because it allegedly introduced new matter into the disclosure. Applicants respectfully disagree with the Examiner's position. Nevertheless, Applicants have amended the specification to remove the alleged new matter. Applicants respectfully submit that the Examiner's objection is moot in light of the amendments to the specification.

Rejections under 35 U.S.C. §112, first paragraph

The Examiner rejected claims 32-45 under 35 U.S.C. §112, first paragraph for allegedly failing to comply with both the written description and enablement requirements. The Examiner asserts that the specification does not provide written description or enable "species of interest", which includes all life forms including viruses.

Applicants respectfully disagree with this rejection, but have amended claims 32-45 in order to expedite prosecution to include methods for identifying single nucleotide polymorphic sites in the genome of mammals. However, Applicants reserve the right to prosecute the subject matter canceled in the claims in a continuation application.

Applicants submit the specification provides written description and fully enables the claims. The specification at pages 13-15, page 44, lines 29-36 and Examples 1-6 clearly discloses and enables conducting genetic analysis using SNPs from mammalian DNA. Applicants submit that DNA is DNA regardless of the species. Thus, DNA does not chemically vary between species and the analysis of SNPs should not in any way depend on the source of the nucleic acid molecules. Moreover, the amended and new claims are directed to use of SNPs in genetic analysis of mammalian DNA. These new and amended claims are fully supported by the specification, which teaches the full scope of the claims to one of ordinary skill in the art. Applicants' insight that combinations of SNPs would be extremely useful as genetic markers and can be used for genetic analysis follows from their discovery regarding the distribution and density of SNPs in mammalian genomes. While the illustrative examples in the specification are directed to horse and human studies, one or ordinary skill in the art upon reading the specification would readily understand that the methods and use of SNPs would be applicable to all

species including mammals. Applicants have described this applicability throughout their specification. Therefore, it is respectfully submitted that the specification teaches and fully enables methods for identifying single nucleotide polymorphic sites in the genome of mammals. Accordingly, Applicants respectfully request withdrawal of the rejections.

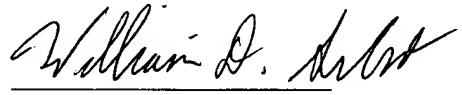
Conclusion

In view of the foregoing amendments, and the remarks set forth above, reconsideration and allowance are respectfully solicited.

Enclosed is the fee for a three-month extension of time ($950.00). No additional fee is believed to be due with respect to the filing of this amendment. If any additional fees are due, or an overpayment has been made, please charge, or credit, our Deposit Account No. 11-0171 for such sum.

If the Examiner has any questions regarding the present application, the Examiner is cordially invited to contact Applicant's attorney at the telephone number provided below.

Respectfully submitted,



William D. Schmidt
Registration No.: 39,492
Attorney for Applicant

Kalow & Springut LLP
Telephone No.: (212) 813-1600

A discussion of the relative advantages and disadvantages of such methods of producing single-stranded molecules is provided by Nikiforov, T. (U.S. patent application serial no. 08/005,061, herein incorporated by reference).

Most preferably, such single-stranded molecules will be produced using the methods described by Nikiforov, T. (U.S. patent application serial no. 08/005,061, herein incorporated by reference). In brief, these methods employ nuclease resistant nucleotides derivatives, and incorporates such derivatives, by chemical synthesis or enzymatic means, into primer molecules, or their extension products, in place of naturally occurring nucleotides.

Suitable nucleotide derivatives include derivatives in which one or two of the non-bridging oxygens of the phosphate moiety of a nucleotide has been replaced with a sulfur-containing group (especially a phosphorothioate), an alkyl group (especially a methyl or ethyl alkyl group), a nitrogen-containing group (especially an amine), and/or a selenium-containing group, etc.

Phosphorothioate deoxyribonucleotide or ribonucleotide derivatives (e.g. a nucleoside 5'-O-1-thiotriphosphate) are the most preferred nucleotide derivatives. Any of a variety of chemical methods may be used to produce such phosphorothioate derivatives (see, for example, Zon, G. et al., Anti-Canc. Drug Des. 6:539-568 (1991); Kim, S.G. et al., Biochem. Biophys. Res. Commun. 179:1614-1619 (1991); Vu, H. et al., Tetrahedron Lett. 32:3005-3008 (1991); Taylor, J.W. et al., Nucl. Acids Res. 13:8749-8764 (1985); Eckstein, F. et al., Biochemistry 15:1685-1691 (1976); Ott, J. et al., Biochemistry 26:8237-8241 (1987); Ludwig, J. et al., J. Org. Chem. 54:631-635 (1989), all herein incorporated by reference). Phosphorothioate nucleotide derivatives can also be obtained commercially from Amersham or Pharmacia.

Importantly, the selected nucleotide derivative must be suitable for in vitro primer-mediated extension and provide nuclease resistance to the region of the nucleic acid molecule in which it is incorporated. In the most preferred embodiment, it must confer resistance to exonucleases that attack double-

Step 6: Computer-Assisted Interpretation of Genotype. The colorimetric data from a number of loci is converted to an SNP genotype for the particular individual tested.

The method is preferably conducted in the following manner:

GBA Template Preparation.

Amplification of genomic sequences was performed using the polymerase chain reaction (PCR). In a first step, one hundred nanograms of genomic DNA was used in a reaction mixture containing each first round primer at a concentration of 2 M and 10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin; and 0.05 units per l Taq DNA Polymerase (AmpliTaq, Perkin Elmer).

To obtain single-stranded template for use with solid-phase immobilized primer, either of two methods may be used. First, the amplification may be mediated using primers that contain 4 posphorothioate-nucleotide derivatives, as taught by Nikiforov, T. (U.S. patent application serial no. 08/005,061). Alternatively, a second round of PCR may be performed using "asymmetric" primer concentrations. The products of the first reaction are diluted 1/1000 in a second reaction. One of the second round primers is used at the standard concentration of 2 M while the other is used at 0.08 M. Under these conditions, single stranded molecules are synthesized during the reaction.

Solid phase immobilization of nucleic acids.

For the GBA procedure, solid-phase attachment of the template-primer complex simplifies washes, buffer exchanges, etc., and in principle this attachment can be either via the template or the primer. In practice, however, especially when non gel-based detection methods are employed, attachment via the primer is preferable. This format allows the use of stringent washes (e.g., 0.2 N NaOH) to remove impurities and reaction side products while retaining the haptenated dideoxynucleotide covalently linked to the 3'-end of the primer.

Therefore, for GBA reactions in 96-well plates (Nunc Nunclon plates, Roskilde, Denmark), the GBA primer was covalently coupled

TABLE 6				
Sample No.	CEPH DNA No.	Adsorption at A_{450} Base G	Base A	Genotype
1	1333-10	.100	.556	AA
2	1333-02	.084	.782	AA
3	1333-04	.372	.369	GA
4	1333-05	.081	.905	AA
5	1333-07	.321	.346	GA
6	1333-08	.084	.803	AA
7	1340-09	.675	.092	GG
8	1340-10	.084	.756	AA
9	1340-12	.537	.096	GG
No DNA	N/A	.076	.097	N/A

False Report Rate

In the current study, two types of potential false reports can be encountered due to either (1) PCR failures or (2) incompatibility between the genotype obtained on opposite strands. Only data from those animals which had been successfully typed in both strands was included in the allelic frequency calculations. Sixty horses typed with respect to 18 sites amounts to 1,080 genotypings. 95% of all typing experiments were successful overall. No typing errors were due to traditional PCR failures. 3.8% false reports were encountered at the GBA step either because the PCR was unsuccessful at the single strand step or due to operator error. 1.1% of all typings produced incompatible data between the strands for unknown reasons.

In sum, the GBA (genetic bit analysis) method is a simple, convenient, and automatable method for interrogating SNPs. In this method, sequence-specific annealing to a solid phase-bound primer is used to select a unique polymorphic site in a nucleic acid sample, and interrogation of this site is via a highly accurate DNA

polymerase reaction using a set of novel non-radioactive dideoxynucleotide analogs. One of the most attractive features of the GBA approach is that, because the actual allelic discrimination is carried out by the DNA polymerase, one set of reaction conditions can be used to interrogate many different polymorphic loci. This feature permits cost reductions in complex DNA tests by exploitation of parallel formats and provides for rapid development of new tests.

The intrinsic error rate of the GBA procedure in its present format is believed to be low; the signal-to-noise ratio in terms of correct vs. incorrect nucleotide incorporation for homozygotes appears to be approximately 20:1. GBA is thus sufficiently quantitative to allow the reliable detection of heterozygotes in genotyping studies. The presence in the DNA polymerase-mediated extension reaction of all four dideoxynucleoside triphosphates as the sole nucleotide substrates heightens the fidelity of genotype determinations by suppressing misincorporation. GBA can be used in any application where point mutation analyses are presently employed -- including genetic mapping and linkage studies, genetic diagnoses, and identity/paternity testing -- assuming that the local surrounding DNA sequence is known.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.